First Stage Report

Binding Activated Localisation Microscopy based Superresolution imaging of Amyloid fibre

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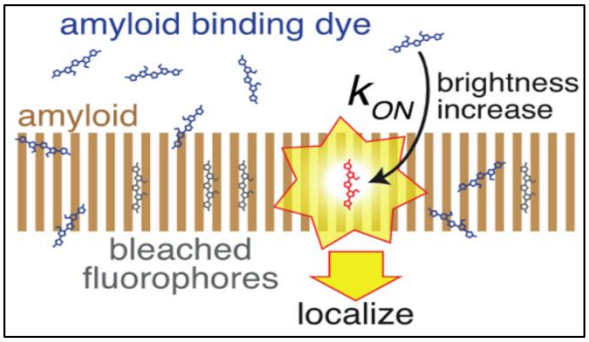
Abstract

For last few decades amyloid fibres are observed as causative agent of various neurodegenerative desises like Parkinson's, Alzheimer's and transmissible Prion etc. What exact physiological environment triggers soluble native proteins to undergo mis-folding and aggregation is still unelucidated primarily due to the lack of crystal formation and insoluble nature of amyloid fibre. This limitations make fluorescence based optical microscopy as one of the most important techniques in the field of amyloid study. Despite of reasonable success in aggregation-disaggregation kinetics and amyloid aggregation inhibitor designing conventional fluorescence based microscopy is fundamentally restricted with diffraction limited resolution of about 200 nm. To overcome this limitation superresolved optical microscopy or nanoscopy is developed in recent years. Some of this techniques including STED, GSD, SSIM uses modified optics to achieve sub-wavelength resolution which makes them experimentally more challenging to set up and expensive. In this review we have demonstrated binding activated localisation microscopy (BALM) to achieve superresolved image of α-Synuclein amyloid fibres based on stochastic binding of Luminescent conjugated oligothiophens (LCO) probe pentamer-formyl thiophene acetic acid (p-FTAA) and subsequent photo bleaching. In, addition this method uses conventional wide field setup to achieve superresolution image of ~ 20 nm spatial resolution, which makes it very relevant to study several aspects of amyloidosis.



Introduction:

Pathological advancement towards curing neurodegenerative disorders including Alzheimer, Parkinson's, transmissible prion diseases has already well-established the cytotoxic behaviour of amyloid fibre[1]. Along with discovery of several functional non-toxic amyloid fibres like melamine deposition, hormonal storage etc[2]. has make the role of amyloid more complex. In last few decades several researches are focussed on amyloid fibres, mechanism of their formation, growth and other aspects primarily in intracellular mimicking invitro condition. In amyloidosis soluble native proteins are observed to mis-folded and aggregated to amyloid fibre under various condition like stress etc[3]. but the exact in vivo influencing agents are still unknown. Amyloid, being the most stable structure of protein folding pathway, makes amyloidosis irreversible and pathogenically fatal[4]. Despites originating from different native proteins having of diverse structure amyloids share a common cross β structure, where β-sheets run perpendicular to fibril's log axis[6]. This infinite β-sheets forms long unbranched ribbon like protofibrils, three to five of which coils around a hollow axis to form the fibril structure. X-Ray crystallographic structure resolution of a small 7 residue fragment of yeast prion protein GNNQQNY has also support cross β structure of amyloid fibre[7].

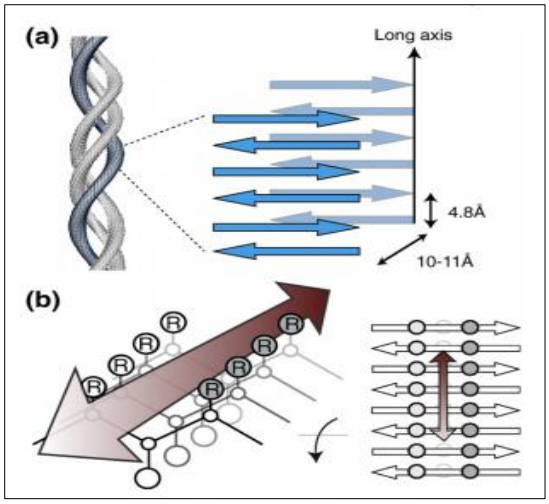


Figure 2: Cross β structure of amyloid fibre (a) amyloid protofibril (b) amino acid chain orients perpendicular to amyloid long axis[5]

However, how amyloid fibre ruptures cell membrane and triggers cell death are still un-elucidated. This is primarily due to their insoluble nature and lack of crystal formation, which make it difficult to study amyloid fibres using conventional methods like single crystal X-Ray crystallography or solution state NMR even with synthetic amyloid fibres[8]. So, Fluorescence base optical spectroscopy, microscopy and imaging combined with electron microscopy, AFM, circular dichrosim (CD) are usually the tools for structural study of amyloid fibre[8]. Amyloid fibres are labelled with amyloid specific chromophore like organic dyes, fluorescence protein e.g. GFP or specifically strained with chromophore labelled antibody in monomer level. This amyloid specific dyes like ThT, ThS, and Nile Red etc. specifically bind to amyloid and become highly fluorescent from mostly non-fluorescent unbound state. There amyloid concentration dependent increase of intensity and shift of emission wavelength maxima on binding has made them excellent method for exhaustively studying aggregation-disaggregation kinetics, there role in diseases and also some steps towards drug designing has progressed in-vitro[9], but exploring molecular mechanism of intracellular interactions with conventional fluorescence microscopy suffers from diffraction limited low resolution of > 250 nm[10,11]. This also makes amyloid morphological analysis impossible. This method also suffers from the probable characteristic change of amyloid fibres during staining.

Stochastic superresolution microscopic methods:

Therefore, advance morphological analysis requires non-traditional super-resolution microscopy or nanoscopy based techniques, which will also be essential to analysis amyloid-cell interaction in molecular level and will pave the way towards invention of new drugs for amyloidosis patients. These techniques are classified into two broad category. STED, GSD, RESOLFT and SSIM etc[12]. involves sophisticated optics to point a LASER beam bellow diffraction point whereas in localisation microscopy point spread function (PSF) of a single emitter is fitted with Gaussian and from the maxima the molecule is precisely located, provided no other molecule is present in the diffraction limited spot. Such a condition is achieved in biological system with a low power LASER light when a small percentage of the fluorophores are stochastically activated in a circle, localised and then photo bleached. Repetition of this process reveals the reconstruction of the super-resolution image. With help of advance computation data acquisition speed can also be greatly increased with molecules of overlapping point spread function. Furthermore no requirement of specialized optics and use of conventional wide field setup make localization microscopy less expensive.

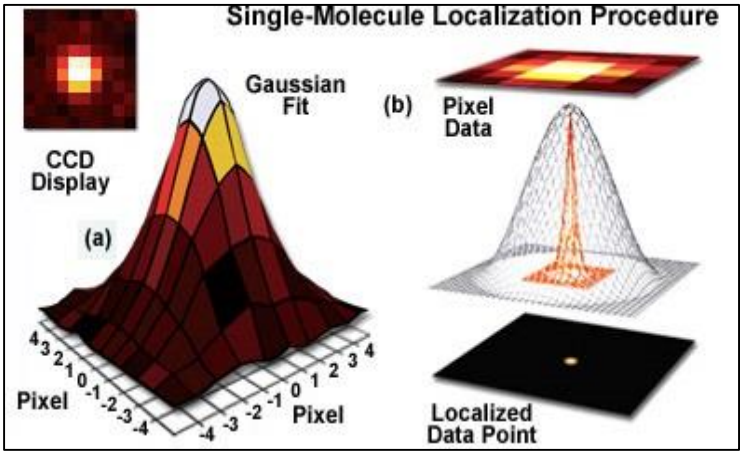


Figure 3: Methods of localisation microscopy (a) Display in CCD and Point-Spread function (b) Fitting of PSI with Gaussian and localisation in sub wavelength resolution [12]

Localisation microscopy has been recently used to study Alzheimer causing Aβ-fibrils, intrinsically disordered α-Synuclein aggregates etc. both in vitro and in vivo with a spatial resolution of ~20 nm. But this techniques requires incorporation of fluorescent dye into amyloid fibre, which suffers from several disadvantages[13]. Bound dye molecule may significantly interfere with the characteristics, structure and function of the amyloid fibres. Background fluorescence from labelled monomer significantly reduces signal to noise ratio and makes it difficult to track amyloid growth over time. Alternatively, immunolabeling increases the ultimate size and changes the topology of amyloid fibres with incorporation of large antibodies ultimately results to significant decrease in resolution[11].

Recently, Luminescent conjugated polyethylene or Luminescent conjugated oligothiophens (LCOs) has demonstrated to preferentially strain and distinguish between amyloid fibres. Asluend A. el al. have well utilised Pentameric Thiophene Derivatives including pentamer-formyl thiophene acetic acid (p-FTAA) as fluorescence specific probe due to their very high fluorescence intensity increase on specific binding with amyloid fibre[14]. Figure 4 shows thousand fold higher intensity of p-FTAA emission spectra on binding with Aβ mature amyloid fibre compared to freshly dissolved peptide. This results to a significant reduction of background noise level leading to high signal to noise ratio.

Finally, to avoid this pre-binding interference, here Binding Activated localization microscopy (BALM) has been used in conjugation with p-FTAA to achieve superresolved image of very

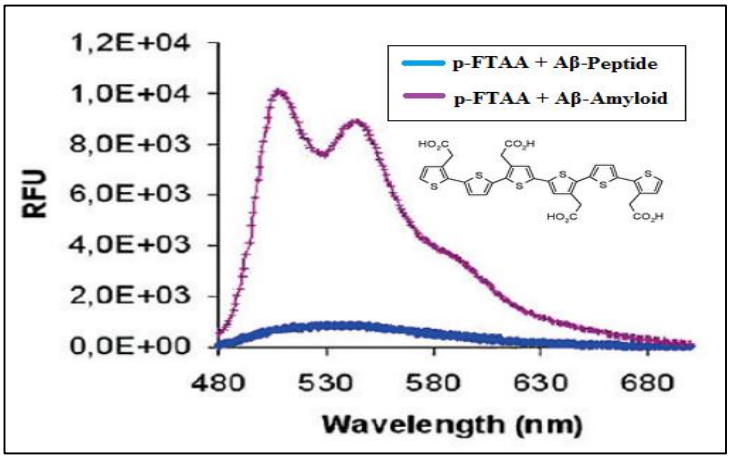


Figure 4: Fluorescence emission spectra of pentamer-formyl thiophene acetic acid (p-FTAA) with freshly dissolved Aβ peptide (blue) and that in presence of Aβ (1-42) amyloid fibre of ~10 µM concentration (magenta) showing large increase of fluorescence intensity[14]

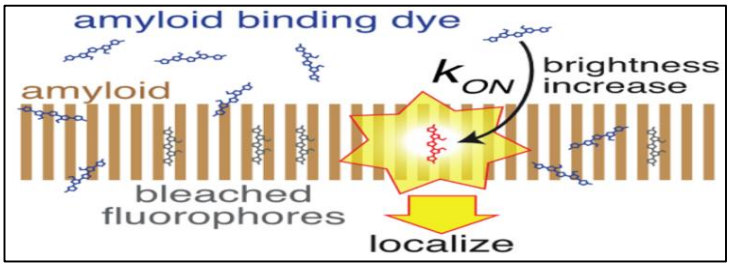


Figure 5: Stochastic binding of dye results a strong fluorescence enhancement[11]

high resolution ~14nm. Here, fluorescent probe molecule is used in nanomolar concentration with amyloid fibre in buffer solution leading to stochastic binding to amyloid fibre and activation. On binding to amyloid fibre specific dyes become highly fluorescent and precisely localized before photo bleaching. New dye molecule rebind to the empty site of the amyloid fibre and repetition of this process ultimately uses to reconstruction of the entire image, unless all binding sites are filled with photo bleached dye molecule. As no prelabelled monomer present in solution noise label is significantly reduced. This method have advantage of no activity or morphology change of amyloid fibre on binding with dye molecule or due to immunolabelling. Moreover, in this method density of localisation is easily controlled by altering the concentration of dye molecule which provides flexibility in acquisition speed and resolution as requirement.

Ease of superresolution imaging of α-Synuclein amyloid fibre:

As previously discussed the fundamental diffraction limits comes into the way of acquiring a good quality image of amyloid fibre. Figure 6 shows how much a conventional microscopy setup blurs α-synuclein amyloid fibre image masking valuable information. Even it can't well separate neighbouring fibres of around 50 nm apart. Figure 6 C and D gives an insights of how much data can be escaped for using a conventional diffraction limited microscopy and urgently demand for a superresolution microscopy.

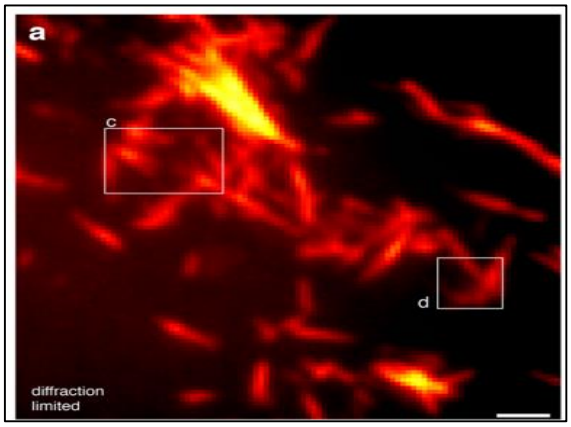


Figure 6: Conventional diffraction limited image of amyloid fibre of alpha Synuclein hardly reviling any structural information[11]

Summary and upcoming future opportunities:

Preceding discussion well establishes the necessity of superresolution microscopy or nanoscopy and concludes BALM as one of the most demanded superresolution imaging technique for studying amyloid fibres. In addition this requires no use of specialised optics and can be performed with conventional wide field setup which makes it operationally more flexible and cost worthy. Primary advantages includes no prelabeling with either fluorescent dye or fusion with large fluorescent protein like GFP and thus allowing to study amyloid fibre in a less altered morphology. Since the LCO probes binds to amyloid fibre only during measurement, the background noise level due to labelled monomer is significantly reduced. Further, BALM is performed in physiologically similar buffer like environment perhaps will be extended to study the invivo interactions of amyloid fibres with organelles and relating amyloid toxicity with such interactions. With BALM based amyloid-organelles interaction study it will be much cheaper to design drugs against fatal neurodegenerative diseases and explore there effect invivo at a molecular level.

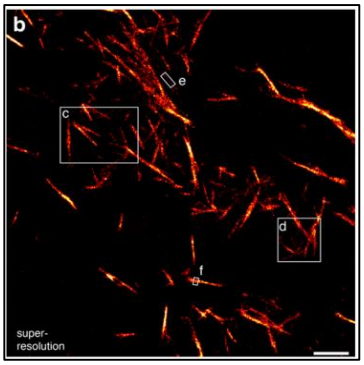


Figure 7: Superresolution Image with BALM[11]

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